A time to remember

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Part One

SCN AND MEMORY PROCESSES

Chapter 2

Periodic Memory Retention Deficits after Active Avoidance Conditioning: A Putative Role for the Suprachiasmatic Nucleus-Vasopressinergic System

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Abstract

In this study, periodic memory retention deficits were shown to persist in continuous light conditions in Wistar rats trained for active shock avoidance (ASA), at training-testing intervals 18 and 30 hours compared to 24 hours. This demonstrates the endogenous nature of these deficits, and the circadian pacemaker (in mammals seated in the suprachiasmatic nucleus (SCN)) as the most plausible generating source. To investigate whether vasopressin, a major output system of the SCN, is involved in this modulation of memory over time, we tested the vasopressin deficient Brattleboro rats and the control Long Evans strain. Brattleboro rats did not demonstrate a deficit at 18 hrs after training, suggesting that AVP is involved in active suppression of memory retention at non-24 h intervals after acquisition of a shock motivated task. In addition, the three strains were compared with respect to explorative behaviour and performance in the shuttle box, and circadian rhythms of wheel running activity. Brattleboro and Wistar rats were found to perform equally well in ASA, and better than Long Evans rats did.

It was concluded that periodic retention deficits, as demonstrated mainly in shock motivated learning tasks, are most probably generated by the circadian system. Furthermore, the authors propose a role for (SCN-) AVP in the modulation of memory retention in time, but not for performance levels per se.

Introduction

The influence of the circadian system on learning and memory processes is receiving renewed interest. Many studies have shown that higher cognitive processing is one of the many complex behaviours that are controlled by the biological clock. For example, performance is subject to circadian variation in rats (Winocur and Hasher, 1999) house mice (Chaudhury and Colwell, 2002) and humans (Kraemer, 2000). Furthermore, amnesia is induced by phase-shifting circadian rhythms in rats (Tapp and Holloway, 1981; Fekete *et al.*, 1985; Devan *et al.*, 2001), house mice (Stone *et al.*, 1992), and humans (Cho *et al.*, 2000).

Compelling evidence for a circadian factor underlying memory processes was found already in the early 70's when a group of researchers published a series of papers on periodic memory retention deficits. The retention of a conditioned response, mostly fear conditioning paradigms, exhibited a maximum directly after training, that recurred at training testing intervals (TTI) with a period of 24 hrs (Holloway and Wansley, 1973a; Wansley and Holloway, 1976). In-between those times retention is worse, and this pattern is independent of the time of day of training (Holloway and Wansley, 1973b). Such multiple retention deficits have been reported both in aversive tasks, i.e. passive (PSA) and active (ASA) shock avoidance, but also in appetitively motivated tasks (Wansley and Holloway, 1975; Hunsicker and Mellgren, 1977). These periodic deficits strongly suggested the involvement of a biological clock, an idea that was substantiated by the finding that lesioning the suprachiasmatic nucleus (SCN), the central circadian pacemaker in mammals, restores good performance at TTI's 18 and 30 h (Stephan and Kovacevic, 1978).

In all these studies, animals were entrained to a light-dark (LD) regimen during training and testing. Therefore, the information on time of day may also have been derived from this Zeitgeber regimen, rather than from endogenous circadian sources. To conclude that the circadian system is the source generating the memory "dips" requires that the phenomenon is independent of imposed LD cycles, and thus persists in constant light conditions. Therefore, the first aim of this study was to investigate whether memory retention dips occur in constant dim red light conditions (DD).

The lesioning study (Stephan and Kovacevic, 1978) raised the question what could be the specific signal from the SCN generating these memory oscillations. A candidate neuropeptide is vasopressin (AVP). AVP is abundant in the SCN of rodents and part of the output pathway. Moreover, AVP was shown to be significantly enhanced in the cerebrospinal fluid (CSF) of rats during a PSA task (Laczi *et al.*, 1984), and is released within the SCN directly after a stressful forced swimming event (Engelmann *et al.*, 1998). Furthermore, we have found an effect on SCN-AVP in response to ASA training in rats (Biemans *et al.*, 2003). The role of AVP in the

control of circadian behaviour is still unclear. Correlative studies have suggested a role for AVP in the organisation of circadian behaviour in house mice (Bult *et al.*, 1993), and voles (Gerkema *et al.*, 1994; Van der Zee *et al.*, 1999a).

The second aim of this study was to see what would happen with periodic memory deficits in the absence of AVP. Manipulation of the AVP system by antisera or selective receptor antagonists could be a way to investigate this. We have opted for the Brattleboro rat (Valtin, 1962), a strain that lacks AVP altogether, due to a single base deletion in the vasopressin gene (Schmale and Richter, 1984). The Brattleboro enabled us to study the role of AVP in memory oscillations without using invasive methods such as intracerebral infusions. In the study we report on two experiments:

1. Memory retention in Wistar rats at multiple training-testing intervals after ASA in constant light conditions;
2. Memory retention at two training-testing intervals after ASA in rats lacking AVP.

Methods

Experiment 1: Memory retention in Wistar rats at multiple training-testing intervals after ASA in constant light conditions.

Animals and housing

Male Wistar rats (n=40) were used, weighing 300-350 g at the time of the experiment. Rats were individually housed in cages (30×45×50 cm) equipped with a running wheel. The rats were housed in a light and temperature controlled climate room. They were entrained to a 12:12 hours light dark (LD) cycle, with lights off at 09:00. Rats were entrained for at least 10 days before the onset of the experiment. The running wheel and a food dispenser, that was suspended outside the cage and activated a switch when a rat obtained food pellets, enabled activity patterns to be monitored throughout the experiment, except during testing. Switches on the running wheel and food dispenser relayed activity signals to a PC-based event recording system (ERS), storing pulses in 2-minute bins. The experiment started on the second day after switching to constant dim red light (DD: ± 1 lux inside the cage).

Apparatus and experimental procedure

The active shock avoidance or shuttle box (Coulbourn Instr., PA, USA) is an automated device that consists of two identical compartments (25×25×30 cm), separated by a low threshold. The walls are made of translucent plastic except for the metal sides. The floor consists of stainless steel bars (0.5 cm), spaced 1.3 cm apart, through which a footshock can be delivered. The apparatus was placed in a sound-attenuated room, dimly lit (± 2 lux) by a red-painted light bulb (25 Watt, Osram).

For training and testing, rats were transported from the climate room to the experimental room in their home cage. They were always placed in the left compartment of the shuttle box facing away from the centre of the cage. Before onset of the first trial, they were allowed to explore for three minutes to habituate to the apparatus. During habituation, the number of spontaneous crossings (Crosshab) between compartments was recorded. A trial started with the presentation of a 4.5 kHz tone (conditioned stimulus (CS)). Five seconds after onset of the CS, a scrambled footshock (unconditioned stimulus (US): 0.3 mA for 3 s) was delivered. After 8 s, the CS + US were terminated, and an inter-trial interval of 30 s started. A jump to the other compartment during the first 5 s of CS presentation terminated the CS and recorded an avoidance. If the rats jumped to the other side during the 3 s presentation of the US, CS + US were terminated and an escape was recorded. If rats failed to change compartments during the 8 s of CS presentation, a "no response" was recorded. During the inter-trial intervals, the number of crossings (Crossiti) was also recorded.

On the second or third day after switching to DD conditions, rats received one training session (30 trials) of active shock avoidance (ASA) between 13:00-19:00 hrs, during their active phase. They were matched on performance after the training session and assigned to one of the following training-testing interval (TTI)-groups: 18, 24, or 30 h. At exactly 18, 24 or 30 hrs after their individual training session, the rats were tested for their retention performance. Care was taken that times of day of training were distributed evenly over the TTI groups. The retention session was identical to the training (30 reinforced trials).

We analysed the following parameters: number of correct avoidances (CAR), trials to a criterion of 3 consecutive avoidances (ttcrit), Crosshab and Crossiti.

Experiment 2: Memory retention at two training-testing intervals after ASA in rats lacking AVP.

Animals and housing

28 male Brattleboro and 30 Long Evans rats were used, weighing 380-570, and 320-545g respectively at the time of the experiment. Slightly older Brattleboro rats (two-three weeks older compared to the Long Evans rats) were used, since Brattleboro rats are growth-retarded (Sokol and Sise, 1973; Arimura *et al.*, 1968), and shock sensitivity is influenced by weight rather than age (Gibbs, 1973). Brattleboro rats completely lack vasopressin (the anti-diuretic hormone, ADH), and therefore the kidneys hardly reabsorb water. This had the important practical consequence that cages had to be cleaned often (every other day). Care was taken to disturb the rats minimally during cleaning. By the time the experiment started, rats had become so accustomed to the cage-cleaning procedure that they would walk to the clean floor plate without handling required. Long Evans rats were housed together with the Brattleboro's in the same climate rooms, so the degree of disturbance due to cleaning was similar. Light schedule and housing conditions in the climate room were identical to that of experiment 1, except that rats were divided over two climate rooms, and could therefore all be trained on the same day after onset of DD (2 days). Rats were entrained for at least 14 days before switching to DD.

Apparatus and experimental procedure

Apparatus and experimental procedures were identical to those of experiment 1, except that there were only two TTI-groups, 18 or 24 h. These times were chosen based on experiment 1, in which the 18 h group showed the strongest effect compared to the 24 h group.

Results

General remarks

In all three strains, experimental rats could be divided in responders and non-responders to the ASA training procedure. Non-responders hardly made any responses (avoidances or escapes) during the training session. Typically, they would freeze in a corner of the shuttlebox, and display "learned helplessness". These rats were not able to acquire the ASA task (within 30 trials). Because our design required a two-tailed response (better, equal or worse than training) a post-hoc arbitrary criterion was set of at least 15 responses (avoidances + escapes). Only data of rats that met this criterion were included in the analysis of TTI retention, which comprised 73% of the Wistars, 73% of the Long Evans and 71% of the Brattleboro's.

The time of training within the time frame of 13:00-19:00 hrs did not affect acquisition rate. Correlations between training order and CARs were absent in all strains (Spearman correlation Wistar: r=0.13; Long Evans: r=-0.26; Brattleboro: r=0.25, p>0.05 in all cases). Also, the number of CARs of rats trained between 13:00-16:00 hrs versus those trained between 16:00-19:00 hrs did not differ (Wistar 10.3±5.2 versus 9.7±6.7; Long Evans: 4.3±4.8 versus 6.0±5.4; Brattleboro: 10.9±7.2 versus 8.3±6.6, p>0.05 in all cases). In case of the Wistar rats there was no effect of number of days (2 or 3) spent in DD on the number of avoidances (10.7±5.5 versus 9.3±6.4, p>0.05), nor an interaction effect between days in DD and training time. Therefore, the data were pooled with respect to the timing mentioned above.

Experiment 1: Memory retention at multiple TTI's after ASA in constant light conditions.

ASA acquisition

Wistar rats displayed significant learning within three blocks of 10 trials of training (RM ANOVA: p<0.001), and reached a level of 54% correct avoidances on average during the last block of 10 trials. Overall, they made on average 10 avoidances and 10 escapes (Table 1). How well Wistars learn, can be predicted from their behaviour during the 3 minute habituation period, since a significant correlation was found between Crosshab and total avoidances (Pearson correlation, r=0.38, p=0.014). Rats that made more avoidances, also made more escapes (Pearson correlation, r=0.45, p=0.003 (Table 1)), in spite of the fact that the sum of avoidances and escapes is restricted to 30.

Table 1. Crossings and responses (avoidances and escapes) during training for the three rat strains (all rats included).

	Spontaneous crossings		Responses	
	Habituation	Inter-trial interval	Avoidances	Escapes
Wistar (1)	7.1 ± 0.46‡	6.2 ± 0.55	10.0 ± 0.94**2	10.3 ± 0.90
Long Evans (2)	9.4 ± 0.94	9.0 ± 0.87‡	5.1 ± 0.93	13.2 ± 0.91*3
Brattleboro (3)	10.5 ± 0.83**1	19.4 ± 2.1**1,2 ‡	9.5 ± 1.3*2	9.4 ± 1.0

Asterisks indicate significant differences (*p<0.05, **p<0.01) between strains(1,2,3,). ‡ Indicates a significant correlation between spontaneous crossings during habituation or inter-trial intervals and number of correct avoidances within one strain.

Memory retention at TTI's 18, 24 and 30 h

11 Wistar rats were omitted from further analysis as they did not meet the response criterion, which left 29 rats, divided over the three TTI's (18 h: n=11, 24 h: n=8, 30 h: n=10). The acquisition curves for these rats (Fig. 1A) did not differ (RM ANOVA, p=0.68) for TTI. Hence, the exclusion of rats did not invalidate the original matching criteria based on which rats were assigned to the different TTI groups. An average of 67.6% correct avoidances was reached in the last block of training.

During retention testing, the TTI 24 h group outperformed the TTI 18 and 30 h groups in terms of the number of CARs (Fig. 1A). A significant effect of TTI is present (RM ANOVA, p<0.01), and post hoc testing revealed significant differences for both TTI 18 h (p<0.01), and TTI 30 h (p<0.05) compared to TTI 24 h. There was no interaction effect of testing block × TTI. The TTI 24 h group needed significantly fewer trials than the TTI 18 h group to reach a performance of 3 consecutive avoidances (Fig. 2A) (MWU, p=0.02). TTI 24 h is also lower than 30 h, though not significantly (p=0.089). Avoidances in the first 10 trials of the retention test (Fig. 3A) were higher in the TTI 24 h group compared to the TTI 18 h group (MWU, p=0.049), but not significantly so compared to the TTI 30 h group (p=0.35).

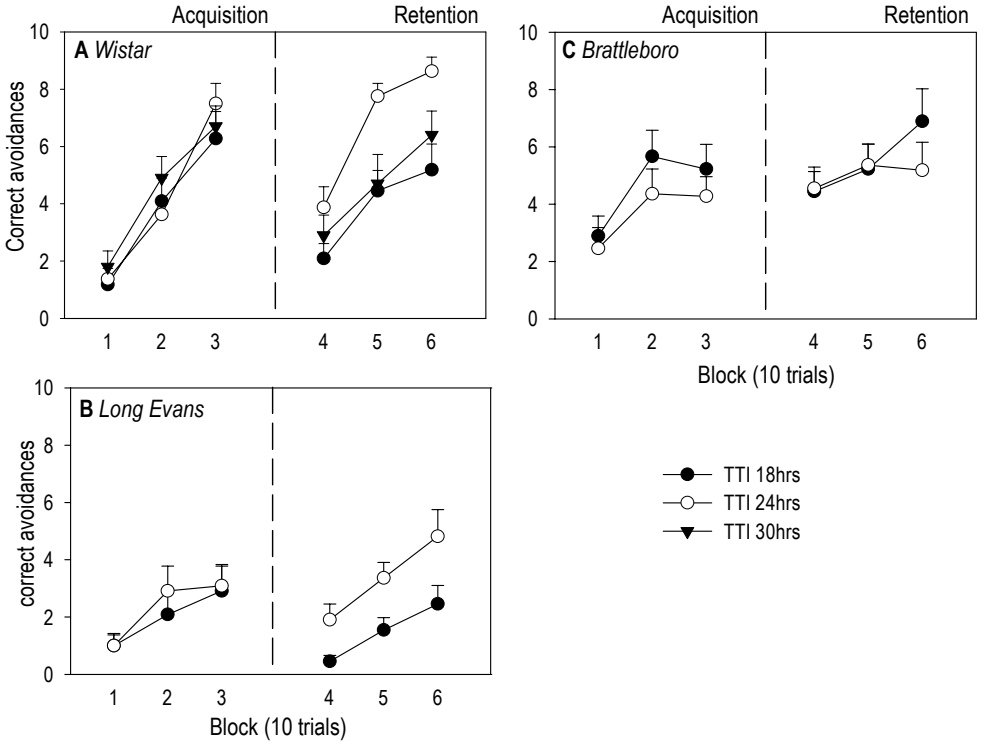

Figure 1. Number of correct avoidances during acquisition (block 1-3), and retention on the following day (block 4-6) at TTI's 18, 24 and 30 h, for Wistar (A), Long Evans (B) and Brattleboro (C) rats. These curves represent the average scores ± S.E.M. for rats meeting the criterion (see text). Wistars: TTI's 18 h: n=11, 24 h: n=8, 30 h: n=10; Long Evans TTI's 18 h: n=11, 24 h: n=11; Brattleboro's: TTI's 18 h: n=9, 24 h: n=11.

Experiment 2: Memory retention at two training-testing intervals in Brattleboro rats.

ASA acquisition

Both Brattleboro and Long Evans rats showed significant learning during acquisition (RM ANOVA, $p<0.001$). The Long Evans rats reached an average of 24.8% correct avoidances in the last block of training, whereas the Brattleboro rats reached 38.9%. Overall, Long Evans made on average 5.1 avoidances during the 30 trials of the acquisition session, and 13.2 escapes (Table 1). Brattleboro's made 9.5 avoidances and 9.4 escapes during training. Brattleboro's made significantly more avoidances (T-test, $p=0.008$), but significantly less escapes (T-test, $p=0.007$) than Long Evans.

For both the Long Evans and the Brattleboro's, learning performance can be predicted from the number of spontaneous crossings made during the inter-trial interval (Pearson correlation Crossiti with total avoidances: Long Evans: $r=0.45$, $p=0.012$; Brattleboro; $r=0.74$, $p<0.001$).

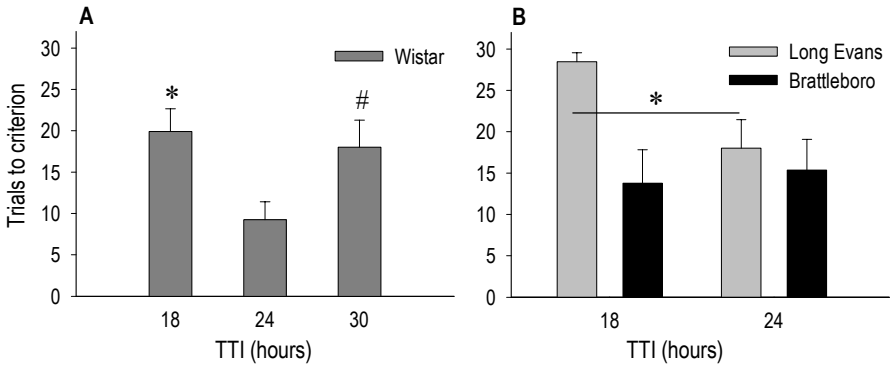

Figure 2. Number of trials needed to reach a criterion of 3 consecutive correct avoidances during retnetion testing at TTI's 18, 24, and 30 h for Wistar rats (A), and at TTI's 18 and 24 h for Long Evans and Brattleboro rats (B). * indicates significant ($p<0.05$) differences between TTI 18 h and 24 h, and # indicates near significant differences($p<0.1$) between TTI 30 h and 24 h.

Memory retention deficits at TTI's 18 and 24 h

8 Brattleboro and 8 Long Evans rats were omitted from further analysis since they did not meet the learning criterion, which left 22 Long Evans (18 h: n=11, 24 h: n=11), and 20 Brattleboro rats (18 h: n=9, 24 h: n=11) divided over the TTI's. Acquisition and retention curves for these rats are displayed in Fig. 1B and 1C, respectively. Acquisition did not differ for TTI 18 and 24 h both in case of the Long Evans (RM ANOVA, $p=0.66$) and Brattleboro ($p=0.34$) rats, indicating adequate matching after omission of rats.

For the Long Evans, the TTI 24 h group outperformed the TTI 18 group (Fig. 1B) during retention testing. A significant effect of TTI is present (RM ANOVA, $p=0.013$). There was no interaction effect of Block × TTI. The TTI 24 h group needed significantly fewer trials than the TTI 18 h group to reach a performance of 3 consecutive avoidances (Fig. 2B) (MWU, $p=0.014$). The numbers of avoidances during the first 10 trials of retention testing are displayed in Fig. 3B. Long Evans rats made significantly more avoidances in the TTI 24 h than in the TTI 18 h group (MWU, $p=0.028$).

In contrast, for the Brattleboro rats retention curves for TTI 18 h and 24 h did not differ (RM ANOVA, $p>0.05$), and there was no interaction between TTI × Block ($p>0.05$). Neither the number of trials to a performance of 3 consecutive avoidances (Fig. 2B) (MWU, $p>0.05$), nor the number of avoidances (Fig. 3B) during the first 10 retention trials (MWU, $p>0.05$) differed for TTI 24 h compared to 30 h group.

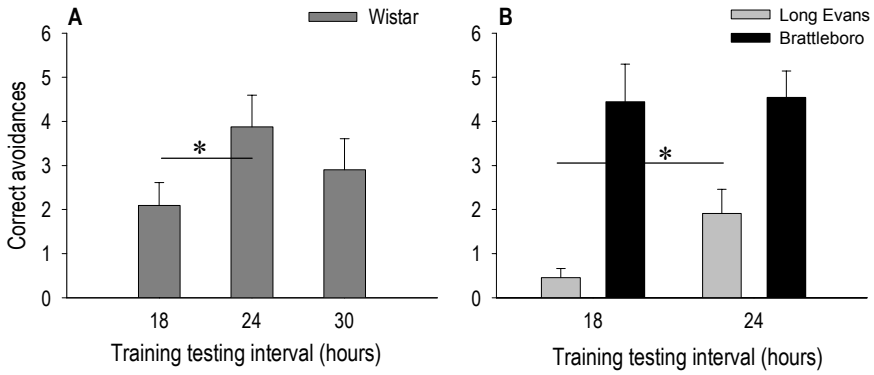

Figure 3. Number of correct avoidances in first block of 10 trials during retention testing at TTI's 18, 24 and 30 h in case of Wistar rats (A), and at TTI's 18 and 24 h in case of Long Evans and Brattleboro rats (B). * indicates significant difference ($p<0.05$) between TTI 18 h and 24 h.

Strain comparisons

Strain differences clearly exist in ASA learning when taking all rats (i.e. including the ones that did not meet the criterion for retention analysis) into account (Table 1). Kruskal-Wallis ANOVA revealed a significant effect of strain on total number of avoidances ($p<0.01$) and escapes ($p<0.05$). Long Evans rats did not learn the ASA learning task as rapidly as the other strains. They made significantly less avoidances than Wistars (Tukey HSD, $p=0.003$), and Brattleboro's ($p=0.017$). Long Evans rats made more escapes than Brattleboro's (Tukey HSD, $p=0.024$), but not significantly more than the Wistars did ($p>0.05$). The explorative behaviour (crossings during habituation and during the inter-trial intervals) also differed (Kruskal-Wallis ANOVA on Crosshab and Crossiti: $p<0.001$). Brattleboro's made the most crossings, during both habituation and inter-trial intervals. They differed significantly from the Wistars during both (Tukey HSD, $p=0.003$ and $p<0.001$, respectively), and from Long Evans during the inter-trial intervals ($p<0.001$).

All rats were entrained to the LD 12:12 hrs cycle at the onset of the experiment. Freerunning periods deviate little from 24 h in the first few days after the transition to DD (Pittendrigh and Daan, 1976), so retention testing always occurred within ± 15 minutes of the circadian time that the rats were trained. When comparing the different strains of rats during an LD period, Brattleboro rats were the most active in the running wheel (Fig. 4), whereas Wistar rats made the lowest number of wheel revolutions. Brattleboro rats displayed the most pronounced day-night distribution of activity.

Within strains of rats, the average number of daily wheel revolutions during five days of entrainment prior to the experiment correlated neither with spontaneous crossing behaviour in the shuttle box (Crosshab or Crossiti), nor with the number of

avoidances during the training session (data not shown). This indicates that general activity as measured by wheel running behaviour is not a facilitating factor in the acquisition of an active shock avoidance task.

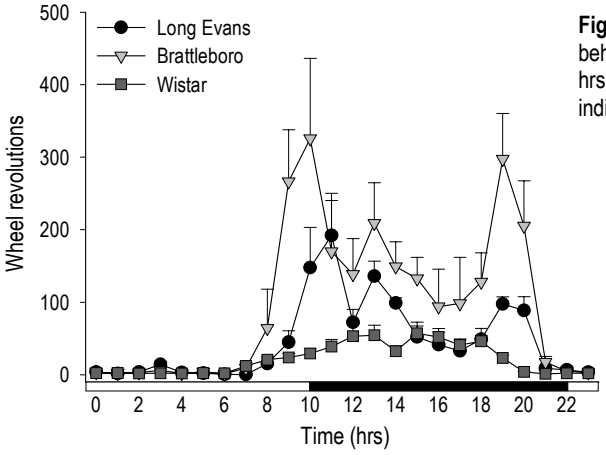

Figure 4. Daily patterns of wheel running behaviour in entrained conditions (LD 12:12 hrs) for the three rats strains. Black bar indicates dark period.

Discussion

Memory retention deficits persist in constant light conditions at TTI's 18 and 30 h after ASA learning as compared to 24 h. Periodic retention deficits have previously been demonstrated in rats in several learning tasks under entrained conditions (Holloway and Wansley 1973a,b; Hunsicker and Mellgren, 1977; Wansley and Holloway, 1975; 1976). This might have been dependent on the day-night transitions as a cue. We showed that deficits at 18 and 30 hrs after ASA training also occur in rats under freerunning conditions, and are thus independent of external LD cycles. It substantiates the endogenous nature of the periodic deficits, and strengthens the idea of the biological clock as an intervening variable or controlling factor in cognitive processes.

A role for the circadian timing system in higher brain functioning has been suggested before (Sylvester *et al.*, 2002; Schwartz *et al.*, 1983). The SCN sends the majority of its efferent fibres to the medial hypothalamus, including the hypothalamic paraventricular nucleus (PVN) (Watts and Swanson, 1987; Watts *et al.*, 1987; Stephan *et al.*, 1981), the starting point of the hypothalamo-pituitary-adrenocortical axis. In addition, the (dorsomedial) SCN projects to the paraventricular nucleus of the thalamus (PVT) (Watts and Swanson, 1987; Watts *et al.*, 1987; Morin *et al.*, 1994; Kawano *et al.*, 2001). The PVT is part of a central circuit

activated during stress and arousal, and innervates multiple limbic structures like the amygdala and nucleus accumbens, as well as cortical regions including the medial prefrontal cortex (mMFC) (Berendse and Groenewegen, 1991; Moga *et al.*, 1995). Recently, a viral transneuronal tracing study has elucidated that the infralimbic part of the prefrontal cortex is innervated by the SCN, a projection dependent on a relay in the PVT (Sylvester, *et al.*, 2002). The SCN could is therefore probably part of a larger circuit affecting functions concerned with stress, arousal and motivation.

Many of these SCN efferent projections contain AVP (Hoorneman and Buijs, 1982). Effects of SCN-AVP on higher brain areas could be accomplished through synaptic contacts, or via diffusion by the CSF. Whether CSF AVP substantially contributes to AVP levels in these target areas, remains to be investigated. The extensive evidence for projections from the SCN to the thalamus suggests a role for neuronal projections as a pathway through which AVP signals can be relayed. Since AVP is such a major output system of the SCN, it is a candidate neurochemical signal for generating memory oscillations. Moreover, AVP has historically been linked to learning and memory processes (for excellent reviews see: Sahgal, 1984; Koob *et al.*, 1989; Engelmann *et al.*, 1996; De Wied, 1997). The focus on AVP research in this respect has been on major AVP producing hypothalamic sources: the magnocellular neurones of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. SCN-AVP has been considered by Laczi *et al.* (1983), who found a depletion of AVP immunoreactivity (ir) in the SCN after PSA.

To test the hypothesis of AVP as a putative causal factor for (periodic) memory deficits we used the Brattleboro rat, lacking endogenous AVP because of a single base mutation in the gene encoding AVP (Schmale and Richter, 1984). We found that Bratttleboro rats had no memory retention deficit at 18 hrs after ASA training, in contrast to the control Long Evans strain, and Wistar rats, suggesting a role for AVP in the circadian regulation of memory. The Brattleboro rats used in this study had normal circadian rhythms of wheel running and feeding behaviour, both in LD and DD conditions, in agreement with earlier studies (Groblewski *et al.*, 1981; Peterson *et al.*, 1980; Brown and Nunez, 1989). This demonstrates that although vasopressin is a major SCN-output system, it is not a prerequisite for the generation or maintenance of circadian behavioural rhythms per se, at least in rats. Nonetheless, AVP might play a role in other processes that are under circadian control, for example the circadian regulation of memory processes.

Reports on AVP involvement in memory processes date back to 1965, when De Wied and co-workers (1965) found that posterior pituitary lobectomy impaired the acquisition and retention of a conditioned response, and later pinpointed vasopressin as the main component causing these deficits (De Wied, 1971). Since then, many publications addressed the issue. Conflicting results were found (see above-mentioned reviews), and the matter is still under debate. The Brattleboro rat

became a popular model for studying the intrinsic effect of AVP on learning and memory. Numerous studies on Brattleboro rats, however, do not unequivocally support a role for AVP (review: Ambrogi Lorenzini *et al.*, 1991). The alleged memory deficits in Brattleboro rats have also been attributed to disturbed circadian sleep patterns, and especially the reduction of paradoxical sleep (PS) (Danguir, 1983), and increased nocturnality of PS and slow wave sleep (SWS) (Brown and Nunez, 1989). Brattleboro rats compromise their sleep by the need to drink almost continuously, and disturbance of paradoxical sleep is known to impair memory function (Fishbein *et al.*, 1971; Stern, 1970). Danguir showed that normal sleep patterns (i.e. the restoration of PS to control (Long Evans) levels) were restored after infusion with AVP but also after water infusion, thus taking away the increased drink need, and, possibly the memory deficits.

In this study, we have found no deficit in memory of the Brattleboro rat. In fact, they performed better than the Long Evans in the ASA task during acquisition and retention, and as good as the Wistar rats did. Other researchers have also reported superior performance (Bailey and Weiss, 1978, 1979) or at least no deficits (Carey and Miller, 1982; Celestian *et al.*, 1975; Williams *et al.*, 1983) in Brattleboro rats compared to Long Evans, or Wistar rats. This argues against a role for AVP in the level of learning or memory performance.

We hypothesise that AVP is involved in the timing mechanism from the SCN alerting an individual at the time of day when -on a previous day- something of impact happened. The Brattleboro is in fact a "sick" rat, and many other secondary processes could be responsible for the behavioural differences besides AVP, for example the aforementioned sleep disturbances. Nevertheless, several lines of evidence now suggest that the SCN-AVP system is involved in signalling to brain areas involved in learning and memory, as consequence of a (stressful) learning events (De Wied *et al.*, 1971; Laczi *et al.*, 1983, 1984; Engelmann *et al.*, 1998; Biemans *et al.*, 2003). Moreover, we have recently found a stress-induced AVP fluctuation in the SCN of house mice (Biemans *et al.*, submitted for publication). An AVP oscillation superimposed on the normal circadian AVP release, running independently yet phase-locked to the master clock, might explain the finding that retention is optimal 24 hrs after learning, independent of time of training.

The data from the Brattleboro's in this study suggests that AVP may be a prerequisite for memory modulation in time, but not for retention performance levels per se. In fact, the presence of AVP seems to inhibit retention, since Brattleboro's have good performance at TTI 18 h. Interestingly, injection of an AVP antagonists removes the memory deficit at 6 hours present in normal rats (Le Moal *et al.*, 1981). Likewise, lesioning the SCN produces restoration of retention levels at 18 and 30 hours after PSA training to levels of sham lesioned rats at TTI 24 h (Stephan and Kovacevic, 1978). Finally, we have recently found that a reduced circadian

organisation leads to the absence of periodic memory deficits in aged rats tested repeatedly in a PSA task, whereas they were present in the young controls (Biemans *et al.*, submitted). Hence, the SCN may suppress retention at non-24 h intervals, and this suppression is temporarily released at the 24 h times. General inhibitory actions are common to SCN neurones, as γ-aminobutyric acid (GABA) is the principal neurotransmitter of the circadian system (Moore and Speh, 1993). Nearly all neurones of the SCN are GABA producing, and GABA is probably co-localised with all other peptides in the SCN, including AVP. Therefore, it is likely that SCN neurones utilise both GABA and peptides for innervation of target areas, and that this interaction is largely one of cyclic levels of inhibitory control (Moore and Speh, 1993).

Strain differences in ASA conditioning

Many studies have investigated strain differences in (active) avoidance behaviour, and found that the choice for a particular rat model can severely influence experimental outcome. Several studies have compared the same strains as used here. We found that Wistar rats were best at acquiring the two-way avoidance learning task, whereas Long Evans were worst, and Brattleboro's intermediate. This is in corroboration with several studies stating that albino's outperform pigmented rats in a one way and two way active avoidance design (Ambrogi Lorenzini *et al.*, 1987).

It is known that enhanced handling increases exploration while reducing anxiety, and has a positive effect on learning (Doty, 1968; Fernandez-Teruel *et al.*, 1991a; Kelley, 1993; Lapin, 1995), and Brattleboro rats did get more attention than Long Evans rats in the sense that they were cleaned more often. However, since handling rats in the weeks before a behavioural test is a standard procedure in our laboratory, and cleaning required a minimum amount handling of the Brattleboro rats, this effect is probably small.

We did find that Brattleboro rats made more spontaneous crossings than Wistar and Long Evans rats, especially during the inter-trial interval. This indicates a higher state of arousal and different response to novelty. The idea that Brattleboro rats have a "higher emotionality", was proposed by Brito (1983), who found that homozygous Brattleboro rats adapted more slowly to novel environments, but otherwise performed as well as their AVP containing counterparts. He suggested that the reported memory deficits might be secondary to these altered temperamental dispositions. Others have reported similar habituation, but slightly higher initial open field activity, attributed to a possible altered motivational or attentional state (Williams *et al.*, 1983). The numerous crossings during the inter-trials intervals were advantageous for the Brattleboro rats in the sense that the more crossings a rat made, the better its acquisition. This was not a general finding because the Wistar rats made

much less crossings during the inter-trial intervals and performed just as well. Also, within the Wistar rats there was no such correlation.

As shown in Fig. 4, Brattleboro rats have normal wheel running activity, with a slightly bimodal pattern, similar to Long Evans rats, whereas Wistar rats show a more unimodal patter of behaviour. This in agreement with Wollnik (1991) who showed bimodal patterns of behaviour in hooded strain of rats, and unimodal in an albino strain. Furthermore, it shows again that AVP is not necessary for the generation of endogenous rhythms in behaviour, consistent with several other studies (e.g. Groblewski *et al.*, 1981).

Concluding remarks

In conclusion, we have found that memory deficits persist in the absence of cues about time of day, substantiating their endogenous nature. Furthermore, memory deficits were absent in the AVP lacking Brattleboro rat, suggesting that AVP plays a suppressing role on memory on non-24 h intervals. It was shown again here that the Brattleboro rat does not suffer from learning or memory disturbances, and that its circadian pattern of behaviour is comparable to other strains, containing AVP. We propose that AVP is part of the pathway that controls and mediates information about circadian time to higher brain areas such as the mPFC. Microdialysis would be valuable tool to search supporting evidence for this hypothesis. In addition, it can be concluded that SCN-AVP is not necessary for memory performance, but only for the modulation of memory over time.